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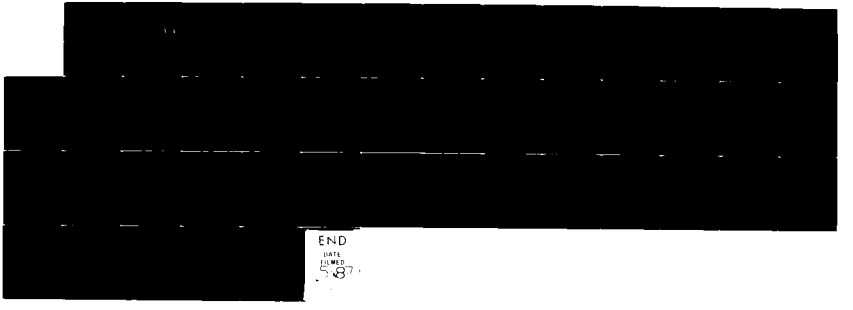

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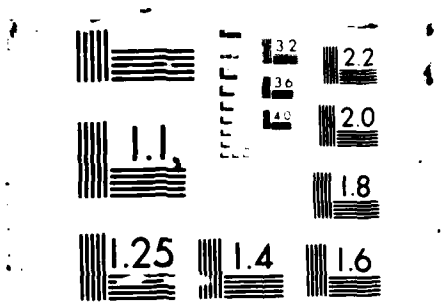

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Chemical Preparation Laboratory for IND Candidate Compounds

Annual Report

by

E.M. Schubert, Ph.D.

January 30,1987

(January 17, 1986 - January 16, 1987)

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT
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17. COSATI CODES		
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06	03	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Nucleosides, antivirals, selenazoles, triazoles, alkaloids, formycin, nucleosides, ribavirin

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During the reporting period eleven compounds were prepared and submitted: 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride; methyl-4-chloro-5-(2,4-dichlorophenyl)-1(H)pyrazole-3-carboxylate; 3-deazauridine; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin); 4-imino-pyrazolo[3,4-d]-thiazine-6(7H)thione; 7-carbamoyl-1-β-D-ribofuranosylimidazo-[1,2-b]pyrazol-6-yl-methylsulfone; Lycoricidine triacetate; 2-β-D-ribo-furanoylselenazo-4-carboxamide; 2',3',5'-tri-O-acetyl-2-β-D-ribofuranosyl-selenazo-4-carboxamide; 4H-r,1H-trans,2H-cis,10bH-trans,1-(2'-tetrahydro-pyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)phenan-thridone; 4aH-r,1H-trans,2H-cis,10bH-trans-1,2-Dihydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)phenanthridone. Three compounds remain under investigation: 3-Deazaguanine; Combretastation.

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I. SUMMARY

The syntheses of fourteen target compounds have been examined during the past year. The preparation of eleven of the target compounds had been completed, and the compounds were transferred to USAMRIID, Department of Antiviral Studies. The syntheses of the three remaining target compounds, one of which is unreported in the chemical literature, are being further investigated.

The following target compounds were synthesized during the reporting period: 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride; methyl-4-chloro-5-(2,4-dichlorophenyl)-1(H)pyrazole-3-carboxylate; 3-deazauridine; 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Ribavirin); 4-imino-pyrazolo[3,4-d]-thiazine-6(7H)thione; 7-carbamoyl-1-β-D-ribofuranosylimidazo[1,2-b]pyrazol-6-yl-methylsulfone; Lycoricidine triacetate; 2-β-D-ribofuranoyslselenazo-4-carboxamide; 2',3',5'-tri-O-acetyl-2-β-D-ribofuranosylselenazo-4-carboxamide; 4H-r,1H-trans,2H-cis,10bH-trans,1-(2'-tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)phenanthridone; 4aH-r,1H-trans,2H-cis,10bH-trans-1,2-Dihydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)phenanthridone.

The syntheses of the following target compounds remain under investigation, and their preparations are progressing: 3-Deazaguanine; Combretastatin.

II. FOREWORD

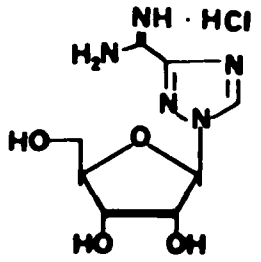
All information in this report is the property of the U.S. Army Medical Research and Development Command. The contractor retains no copyright or patent rights.

All target compounds reported herein were prepared in strict compliance with "Current Good Manufacturing Procedures" (CGMP) guidelines. All intermediates and final products unreported in the chemical literature were fully characterized by elemental and spectral analyses.

IIIa. CUMULATIVE LIST OF COMPOUNDS COMPLETED AND DELIVERED TO U.S. ARMY MEDICAL RESARCH INSTITUTE OF INFECTIOUS DISEASES (USAMRIID)
JANUARY 17, 1986 TO JANUARY 16, 1987

No.	Compound	Amount	Production Control No.
AVS 206	1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride	28.0 g	1256
AVS 332	Methyl-4-chloro-5-(2,4-dichlorophenyl)-1(H)pyrazole-3-carboxylate	25.0 g	1268
AVS DAU	3-Deazauridine	45.0 g	1335
AVS REX	1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide	571 g	1376
AVS 266	4-Iminopyrazolo[3,4-d]-1,3-thiazine-6(7H)thione	15.4 g	1372
AVS 439	7-Carbamoyl-1-β-D-ribofuranosylimidazo-[1,2-b]pyrazol-6-yl-methyl sulfone	12.0 g	1446
AVS 360	Lycoricidine Triacetate	7.1 g	1463
AVS 253	2-β-D-Ribofuranosyl-selenazo-4-carboxamide	36.8 g	1465
AVS 253 TA	2',3',5'-Tri-O-acetyl-2-β-D-ribo-furanosyl-selenazo-4-carboxamide	13.0 g	1596
AVS 360 HP	4H-r,1H-trans,2H-cis,10bH-trans-1-(2',Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetra-hydro-6(5H)phenanthridone	6.4 g	1572
AVS 360 DH	4aH-r,1H-trans,2H-cis,10bH-trans, 1,2-Dihydroxy-8,9-methylenedioxy-1,2-4a,10b-tetrahydro-6(5H)phenanthridone	2.5 g	1571

IIIb. STRUCTURES OF COMPOUNDS SUBMITTED



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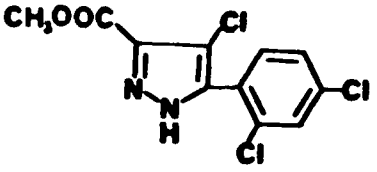

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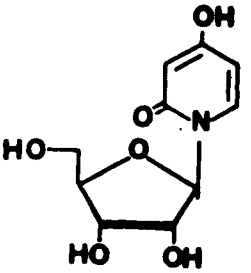

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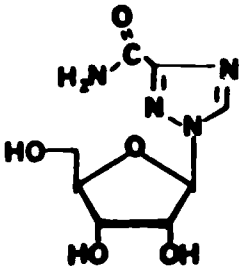
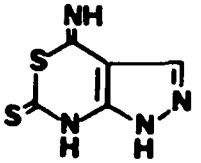


AVS REX



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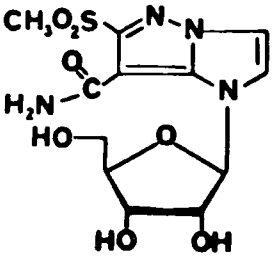

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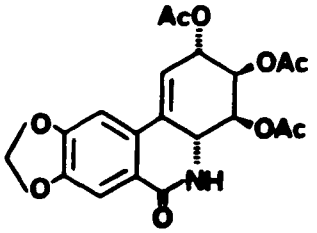

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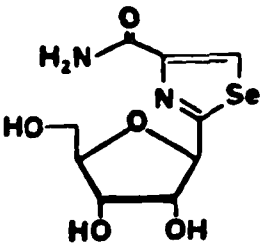

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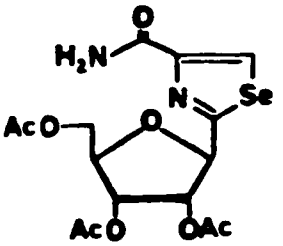
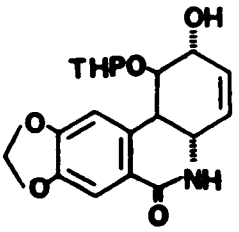


AVS 253 TA



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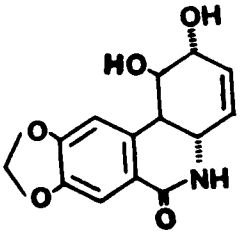
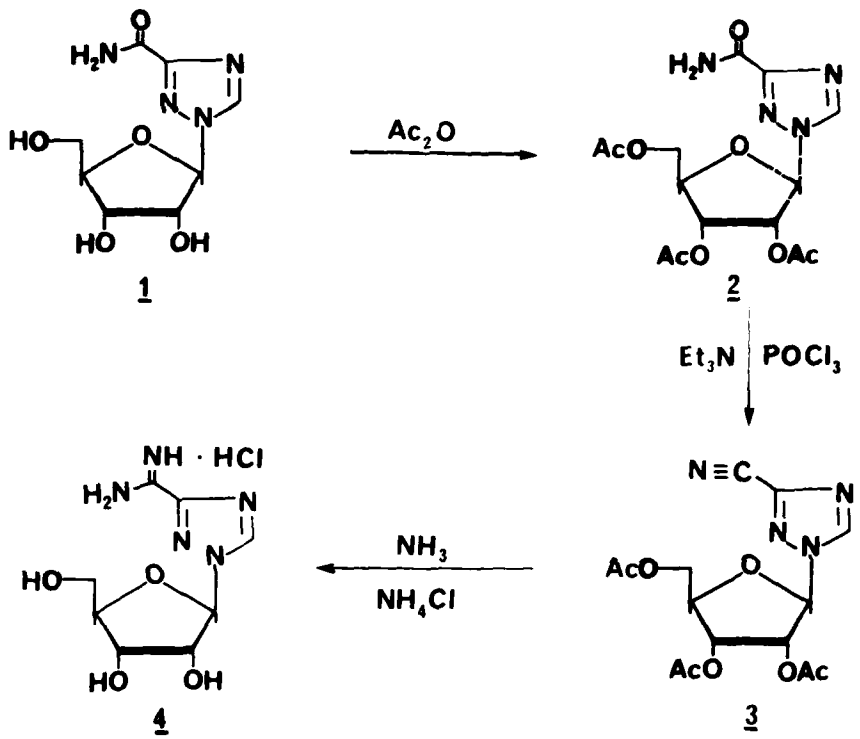


AVS 360 DH

IV. PROCEDURES FOR TARGET COMPOUNDS DELIVERED TO USAMRIID
from January 17, 1986 to January 16, 1987

A. 1-β-D-Ribofuranosyl-1,2,4-Triazole-3-carboxamidine hydrochloride, AVS 206

Synthetic Procedure:



Experimental

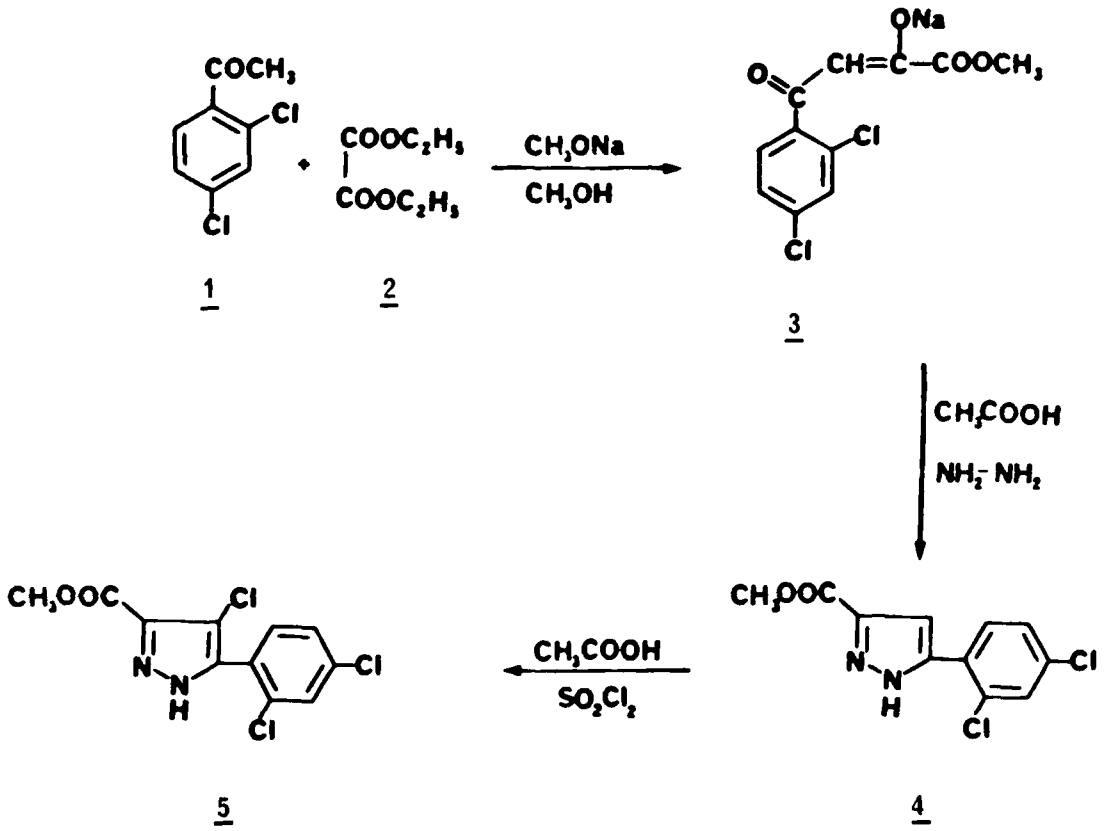
2'3'5'-Tri-O-acetylribavirin (2): A mixture of ribavirin (50 g, 0.2 mol), acetic anhydride (600 mL), and 4,4-dimethylaminopyridine (1 g) is stirred for 60 hours. Unreacted acetic anhydride is evaporated under reduced pressure at 40°. The obtained viscous residue is treated with ethanol (500 mL), and upon evaporation of the solvent the product is dissolved in cold water. The aqueous phase is extracted with ethyl acetate (3 x 300 mL). The combined organic layers are dried over sodium sulfate, and evaporated to give a solid foam which shows as a single spot on TLC. Yield: 75.8 g (100%).

1-(2,3,5-Tri-O-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-3-carbonitrile (3): Tri-O-acetylribavirin 2 (74 g, 0.2 mol) and triethylamine (411 mL, 2.9 mol) are dissolved in chloroform (1200 mL) and cooled to 0°. Phosphorous oxychloride (52 mL, 0.55 mol) is added dropwise over a period of 30 minutes. The reaction mixture is kept at 0° for another 30 minutes, then the ice bath is removed and the reaction is stirred at room temperature for 3 hours. The solvent is evaporated under reduced pressure, then the residue is dissolved in ethyl acetate (1500 mL). The ethyl acetate solution is washed with water (2 x 500 mL) and with saturated sodium bicarbonate solution. The aqueous washings are combined and extracted with ethyl acetate (200 mL), the combined organic layers are dried over sodium sulfate, and treated with charcoal at room temperature. After filtering through a Celite bed the filtrate is evaporated to dryness to yield a colored syrup. The syrup is dissolved in dichloromethane (300 mL) then the solution is passed through a short column packed with silica gel, and eluted with ethyl acetate/dichloromethane 4:1 (500 mL). After evaporation of the solvent a white, crystalline material is obtained which is homogeneous on TLC. Yield: 48.2 g (68%) m.p. 96-98°; lit. 96-97°.

1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamidine hydrochloride (4)[1]: A mixture of the cyanotriazole derivative 3 (47.6 g, 0.135 mol), ammonium chloride (7.3 g, 0.137 mol) and anhydrous liquid ammonia (1000 mL) is kept in a sealed steel bomb at 90° for eighteen hours. Subsequently, the ammonia is allowed to evaporate during a twelve hour period. The residue is dissolved in ethanol (1600 mL), treated with charcoal, and filtered through a Celite bed. The ethanolic solution is concentrated to about 400 mL when the product starts to crystallize. After filtration the mother liquor is concentrated to about 150 mL when a second crop is obtained. The first and second precipitate, found to be identical, are combined, washed with ethanol and ether (100 mL) to give a total yield of 29.3 g (78%) m.p. 179-180°; lit. 177-179°.

B. Methyl-4-chloro-5-(2,4-dichlorophenyl)-1(H)pyrazole-3-carboxylate, AVS-332

Synthetic Procedure:[2]



1 2 3 4 5

AVS-332

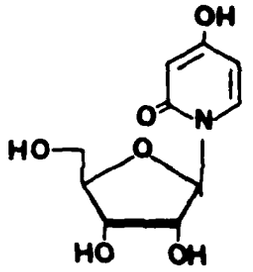
Experimental

Methyl-5-(2,4-dichlorophenyl)pyrazole-3-carboxylate(4): A solution of 2,4-dichloroacetophenone (1) (94.5 g, 0.5 mol) and diethyl oxalate (2) (73.0 g, 0.5 mol) in methanol (100 mL) is added dropwise to a stirred solution of sodium methoxide in methanol, prepared by dissolving sodium metal (11.5 g, 0.5 mol) in 1000 ml of methanol. During the addition, methanol (ca 800 ml) is added to keep the reaction mixture fluid. After the addition is complete the mixture is stirred for 3 hours. The solid is collected by filtration, washed with ethanolic ether (200 mL), ether (200 mL) and dried in air to yield 60.0 g of methyl-2,4-dichlorobenzoylpyruvate sodium salt (3) which is used without further purification. The intermediate 3 (60.0 g, 0.20 mol) is dissolved in glacial acetic acid (550 mL), to which hydrazine hydrate (15.0 g, 0.30 mol) is added over a 5 minute period. The mixture is heated in an oil bath (100°C) for 1.5 h, cooled to room temperature and poured into water (750 mL). After stirring for 30 min. the white precipitate is filtered, washed with hot water (3 x 500 mL) and air dried to give 4 as white powder. Yield 52.0 g (38.4%), m.p. 179-180°C; Lit. m.p. 178-180°C

Methyl-4-chloro-5-(2,4-dichlorophenyl)-1(H)pyrazole-3-carboxylate (5): A mixture of methyl-5-(2,4-dichlorophenyl)-1(H)pyrazole-3-carboxylate (4) (51.2 g, 0.189 mol) and sulfuryl chloride (77 mL, 0.958 mol) in acetic acid (750 mL) is heated in an oil bath (bath temp. 100-110°C) for 3.5 hours. The solution is allowed to cool to room temperature, then it is added to ice-water (1 L). The white precipitate is filtered, washed with cold water, dried in air and dissolved in hot methanol (400 mL). The methanolic solution is treated with charcoal and filtered through a celite bed. The crystallized material is filtered, washed with cold methanol and dried. The filtrate, along with the washings, is concentrated to 150 mL and cooled. The second crop of crystals is filtered, washed with methanol and added to the first crop. TLC of the material shows a second spot. Therefore the material is recrystallized from hot ethanol to give pure 5. Yield 28.2 g (49.2%) m.p. 170-171.5°C; Lit. m.p. 168-172°C

C. 3-Deazauridine, AVS DAU

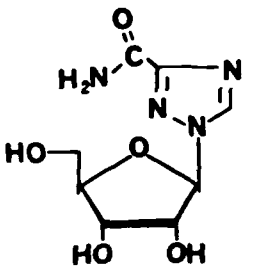
Synthetic Procedure:



3-Deazuridine (48 g, 0.2 mol) is dissolved in 480 mL of boiling methanol. Upon cooling the precipitated material is collected by suction filtration and dried. Yield: 45.5 g (97%), m.p. 234-235°; m.p. lit. 233-235°.

D. 1-β-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide, AVS REX

Synthetic Procedure:



Contaminated ribavirin (694 g) is dissolved in a mixture of methanol (3.6 L) and water (2.25 L) at reflux. Charcoal (10 g) is added, the mixture is kept at reflux temperature for 15 minutes, then filtered through a Celite bed. After standing overnight at 5°, the resulting crystals are collected by filtration and washed with methanol/water.

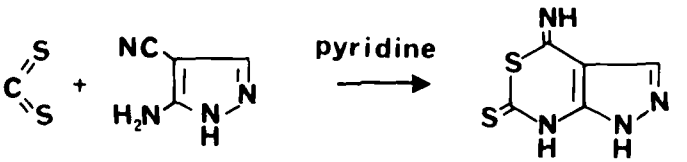
After concentrating the mother liquor, a second crop is obtained, which is shown by analytical evaluation to be identical to the first obtained crop.

Combined yield: 574 g m.p. 166-168°

The second mother liquor is saved to recover more ribavirin for experimental investigations.

E. 4-Iminopyrazolo[3,4-d]-1,3-thiazine-6(7H)thione, AVS 266

Synthetic Procedure:[3]



AVS 266

3-Amino-4-cyanopyrazole (21.6 g, 0.2 mol) is combined with anhydrous pyridine (200 mL) and carbon disulfide (200 mL), and the solution is kept at reflux temperature for 5 hours. Upon cooling, the solvent is evaporated under reduced pressure, the resulting residue is treated with hydrochloric acid 10% (200 mL), and the product is collected by suction filtration. The crystalline material is washed until the washings are neutral, then the solid material is air-dried.

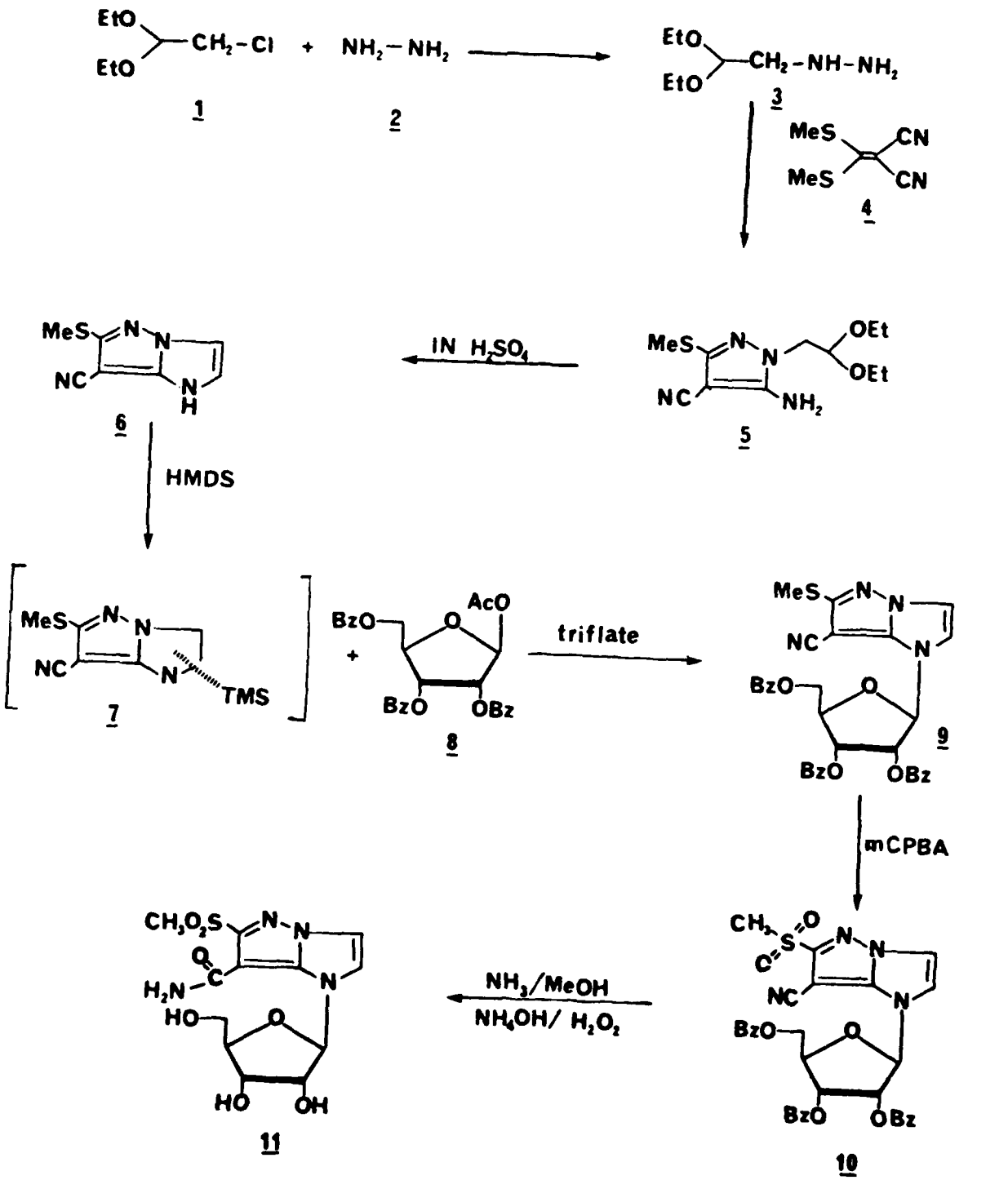
Crude yield: 28 g m.p. > 300°

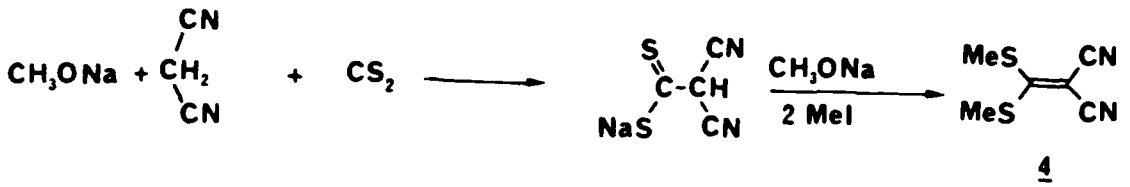
The crude material is recrystallized from a mixture of dimethylformamide (120 mL), ethanol (100 mL) and water (250 mL), and dried in vacuo at 110°.

Yield: 19.2 g m.p. > 300°; lit. > 300°

F. 7-Carbamoyl-1-β-D-ribofuranosylimidazo[1,2-b]pyrazol-6-yl-methyl sulfone, AVS 439

Synthetic Procedure:[4]





4

Experimental

2,2 Bis-methylthio-1-cyano-acrylonitrile (4): Sodium metal (40.2 g, 1.75 mol) is dissolved in methanol (500 ml) and the volume is adjusted to 525 mL with the addition of methanol. 300 mL of the prepared sodium methoxide solution is transferred to a 2 L flask, and the flask is cooled in an ice bath. A solution of malononitrile (66 g, 1 mol) in methanol (50 mL) is added dropwise to the methoxide solution over a one hour period, during which time the formed sodium salt precipitates. Upon additon of methanol (400 mL) and cooling to 5°, carbon disulfide (30 ml) is added dropwise while maintaining the temperature below 10°. More sodium methoxide solution (150 mL) is added and after stirring for 30 minutes, carbon disulfide (15 mL) is added dropwise. Finally the remaining methoxide solution is added, followed by the dropwise addition of carbon disulfide (7.5 mL). The precipitated yellow solid is stirred for 1 hour, then iodomethane (284 g, 2 mol) is added dropwise over a 1 hour period. Stirring is continued for 16 hours at room temperature, then the solvent is evaporated under reduced pressure. The residue is treated with ice cold water (2 L), and the crystalline material that separates is collected by filtration, washed with cold water and recrystallized from ethanol.

Yield: 44 g (26%) m.p. 80°, lit. 81°

Hydrazinoacetaldehyde diethyl acetal (3): A solution of hydrazine (80 g, 2.5 mol) and chloroacetaldehyde diethyl acetal (100 g, 0.68 mol) in ethanol is kept at reflux for 5 hours. After removal of the solvent under reduced pressure, the remaining oil is vacuum distilled to yield the desired product (70-75°/2 mmHg).

Yield: 55.8 g (55.4%)

5-Amino-1-(2,2-diethoxyethyl)-3-(methylthio)pyrazole-4-carbonitrile (5): 2,2-Bis-methylthio-1-cyano-acrylonitrile (65 g, 0.382 mol) and hydrazinoacetaldehyde diethyl acetal (56.5 g, 0.382 mol) are combined in ethanol (600 mL) and stirred for two days. The solvent is evaporated under reduced pressure, and the residue is dissolved in ether (800 mL). The ether solution containing some impurities is filtered, and the filtrate is reduced to a volume of 200 mL. After addition of hexane (400 mL) and swirling the flask to prevent clumping, crystallization starts, and after two hours the white crystals are collected by suction filtration. The mother liquor is evaporated to dryness, and upon treatment with ether (100 mL) and hexane (200 mL) additional product is obtained.

Yield: 75.3 g (73%) m.p. 68-71° lit. 68-69°

6-(Methylthio)imidazo[1,2-b]pyrazole-7-carbonitrile (6): A solution of carbonitrile 5 (72.1 g, 0.267 mol) in sulfuric acid (14 mL conc. acid in 500 mL water) is kept at reflux for 45 minutes. After cooling, the precipitated solid is collected by suction filtration and the obtained product is recrystallized from ethanol (1400 mL).

Yield: 30.8 g (64.8%) m.p. 211-213°

6-(Methylthio)-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazo-[1,2-b]pyrazole-7-carbonitrile (9): A mixture of carbonitrile 6 (8.9 g, 0.05 mol), hexamethyldisilazane (125 mL) and ammonium sulfate (50 mg) are kept at reflux temperature for two hours. Excess HMDS is removed by evaporation and the residue is dissolved in acetonitrile (250 mL). To this solution β-D-ribose-1-acetate-2,3,5-tribenzoate (8) (27.8 g, 0.055 mol) and trimethylsilyl trifluoromethane sulfonate (13.5 mL, 0.07 mol) is added, and the mixture is stirred at room temperature for two days. This reaction mixture is evaporated to dryness and dissolved in ethyl acetate (700 mL). The organic phase is washed twice with 5% bicarbonate solution (800 mL & 400 mL) with brine (2 x 400 mL) and dried over sodium sulfate. After filtering the ethyl acetate solution, the solvent is removed by distillation; the residue is dissolved in dichloromethane (25 mL) and chromatographed on a silica gel column (200-425 mesh, 6 x 55 cm column) using dichloromethane/hexane 1:1 (3 L); dichloromethane/ hexane 3:1 (2 L); dichloromethane (5 L) and dichloromethane/acetone 7:3 as the eluants. Fractions containing the protected nucleoside 9 are combined and evaporated to leave a white solid foam.

Yield: 21 g (67.5%)

TLC: Silica gel/chloroform-ethyl acetate 19:1
Single spot at Rf: 0.39

7-Cyano-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)imidazo[1,2-b]pyrazol-6-yl methyl sulfone (10): To a suspension of carbonitrile derivative 9 (59.1 g, 0.095 mol) in methylene chloride (1950 mL) is added in chlorobenzoic acid (36.1 g, 0.209 mL). The mixture is stirred for 90 minutes, washed with 5% bicarbonate solution (500 mL); the organic layer is dried (sodium sulfate) and evaporated to yield sulfone 10 as a solid foam.

Yield: 62.1 g (99.8%)

7-Carbamoyl-1-β-D-ribofuranosyl-imidazo[1,2-b]pyrazol-6-yl methyl sulfone (11): A suspension of methyl sulfone carbonitrile 10 (61.5 g, 0.094 mol) in methanol (1500 mL) is cooled in a steel bomb to -5°, and ammonia gas is injected for 30 minutes. The vessel is sealed and allowed to stand at room temperature overnight. After venting the bomb, excess ammonia is eliminated by a stream of nitrogen, injected into the reaction mixture. The solution is concentrated to 400 mL under reduced pressure. The solid white precipitate that forms is collected by filtration and washed with ether. From the mother liquor more crystalline material is obtained upon evaporation of the methanol-ether layer.

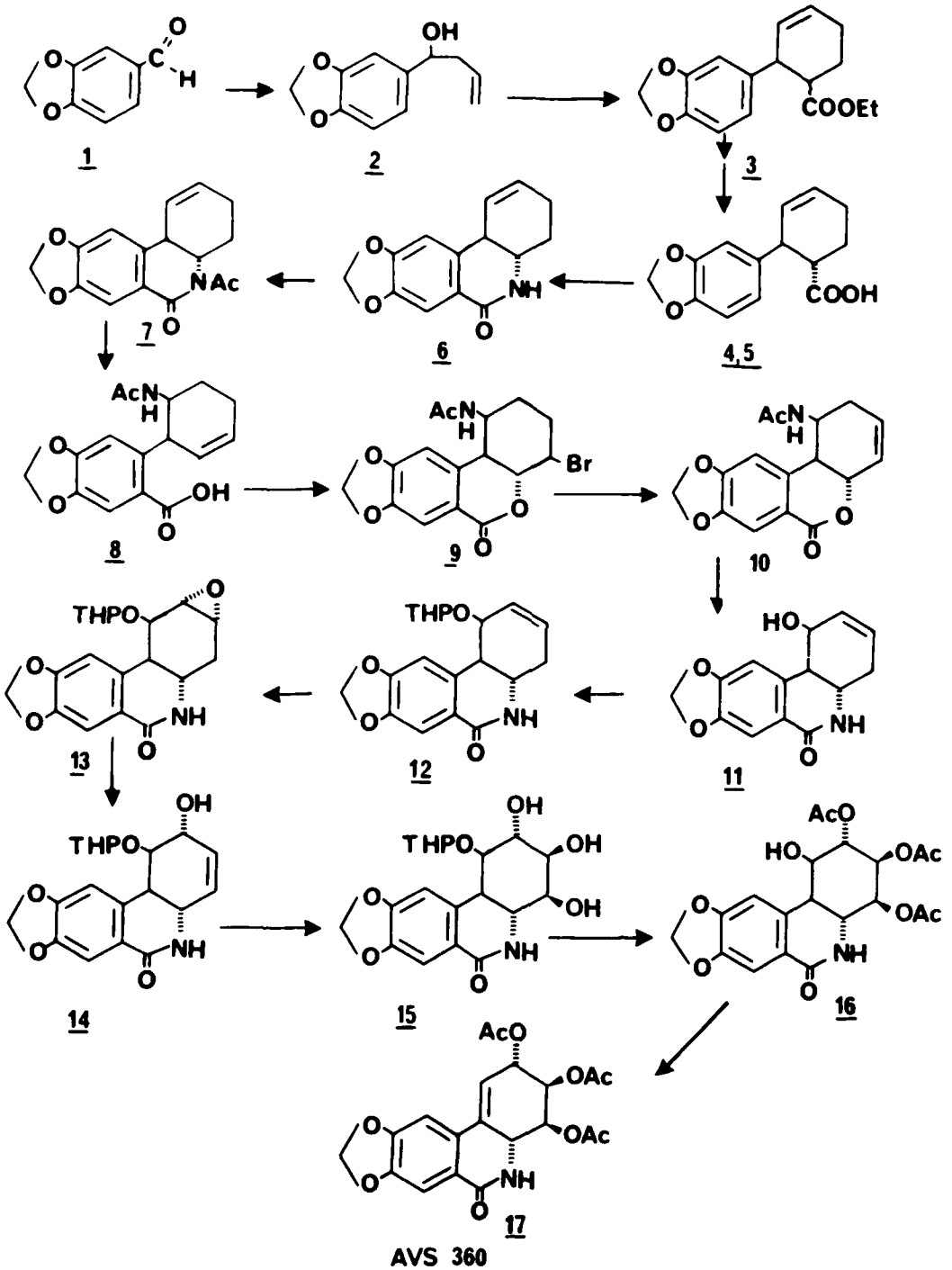
Yield: 28.4 g (88.3%)

The obtained deprotected carbonitrile nucleoside (27.9 g, 0.082 mol) is suspended in 38% ammonium hydroxide (370 mL) and hydrogen peroxide 30% (37.7 mL) is added. After about 4 hours TLC indicates the completion of the reaction and the volume is reduced under diminished pressure until a white solid precipitates. This crystalline material is collected by filtration; the mother liquor is concentrated to yield a second crop of crystals, and upon filtering off this material and checking the identity of the two products, they are combined to give 19.9 g of crude final product. The product is recrystallized from an ethanol (400 mL)/water (40 mL) mixture to give analytically pure nucleoside 11.

Yield: 17.1 g (57.9%) m.p. 189-190° lit. 182-184°

G. Lycoricidine Triacetate, AVS 360

Synthetic Procedure:[5,6,7]



Ethyl-trans-2-(3',4'-methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (3): Piperonal (500 g, 3.33 mol) is dissolved in tetrahydrofurane (3 L), and the solution is cooled below 5° in an ice bath. Allylmagnesium chloride solution (2000 mL, 4.0 mol) is added over a 2½ hour period while maintaining the temperature below 5°. After completion of addition, the reaction mixture is stirred an additional 30 minutes at low temperature. Then it is allowed to warm up to room temperature and kept there for three hours.

After cooling again below 5° a saturated ammonium chloride solution (1 L) is added during a 40 minute interval. The organic layer is separated and washed with brine (4 L) several times, and the aqueous layer is washed with chloroform (1 L). The combined organic layers are dried over sodium sulfate, filtered, and the solvent is removed under reduced pressure. Upon eliminating any remaining solvent under high vacuum, the resulting oil is applied in the subsequent reaction without further purification.

The obtained allyl carbinol derivative 2 (309 g) ethyl acrylate (195 g) and p-toluene sulfonic acid (54 g) are kept in a sealed bomb at 175-185° for six hours. Upon cooling the reaction mixture is submitted to distillation under reduced pressure to eliminate excess ethyl acrylate. The residue is dissolved in ether (3 L), the organic layer is washed with water (1 L), 5% sodium bicarbonate solution (1 L), and water (1 L). After drying with sodium sulfate overnight the solvent is taken off under reduced pressure and the residual oil is fractionally distilled where the desired product 3 boils at 145-155°/0.25 mm Hg, yield 263 g (90%). (Overall yield 29%)

trans-2-(3',4'-Methylenedioxyphenyl)-3-cyclohexene-1-carboxylic acid (4): A solution of sodium metal (10 g, 0.435 mol) in ethanol (300 ml) is combined with a solution of phenyl ester 3 in ethanol (300 mL), and the resulting mixture is kept at reflux for 2 hours. Water (60 mL) is added to the reaction mixture and reflux is continued for 4 more hours. The ethanol is evaporated under reduced pressure, the residue is dissolved in water (1 L) and washed with ether (3 x 300 mL). Upon cooling the aqueous layer, the pH is adjusted to pH 2 with conc. hydrochloric acid (prox. 25 mL), stirred for 30 minutes, and the precipitate is collected by filtration. The product is washed with water and air-dried to yield 81.1 g (90.3%) of acid 4; m.p. 101-102°, lit. 102-103°.

4aH-r,10bH-trans-8,9-Methylenedioxy-3,4,4a,10b-tetrahydro-6(5H)-phenanthridone (6): Carboxylic acid 4 (200 g, 0.81 mol) is dissolved in acetone (1 L), water (200 mL) and triethylamine (82 g, 0.81 mol) is added, and the mixture is cooled in an ice bath. A solution of ethyl chloroformate (87.4 g, 0.81 mol) in acetone (200 mL) is added slowly while stirring. Two hours after completion a solution of sodium azide (80 g, 1.23 mol) in water (200 mL) is added slowly during a one hour interval, followed by two hours of stirring at the lowered temperature. A mixture of toluene (1 L) and water (1.5 L) is added and stirring is continued for an additional 1.5 hours. The organic layer is separated, the aqueous layer washed with toluene (300 mL), and the combined organic layers are washed with water (2 x 1 L) and dried over sodium sulfate.

After filtering, the obtained solution is concentrated to 1700 mL under diminished pressure at 45°, followed by maintaining the concentrated solution at reflux for 4 hours. When TLC showed the disappearance of starting material the solution is cooled, and the solvent is evaporated under reduced pressure to leave 5 as a light tan-colored oil, which is used in the next step without further purification.

4aH-r-10bH-trans-5-Acetyl-8,9-methylenedioxy-3,4,4a,10b-H-tetrahydro-6(5H)phenanthridone (7): A mixture of tetrahydrophenanthridone 6 (50.0 g,0.206 mol), acetic anhydride (500 mL) and DMAP (1.0 g) is refluxed for 4 hours. Upon cooling a solid precipitates from solution. The precipitate is collected by filtration and sucked to dryness. The filtrate is evaporated under reduced pressure (40°), the resulting solid is triturated with ethanol, filtered, and the filter cake is combined with the one obtained previously. After an additional trituration of the combined product with ethanol, the solid is filtered off, washed with ice-cold ethanol and air dried. Yield: 40.0 g (62%); m.p. 155-157°, lit. 157-158°.

4H-r,3H-trans,3-(3',4'-Methylenedioxy-6'-carboxyphenyl)-4-acetamino-1-cyclohexene (8): N-acetylphenanthridone 7 (60.0 g, 0.2 mol) is suspended in methanol (1 L), then a solution of potassium hydroxide (22.4 g, 0.4 mol) in water (400 mL) is added, and the flask is immersed in a water bath preheated to 80°. The reaction mixture reaches a temperature of 70°C, and the reaction is kept at that temperature for 15 minutes. When cooled to room temperature a white solid starts to separate. The volume of the reaction mixture is reduced to 400 mL and the resulting precipitate is collected by filtration, washed with water (2 x 25 mL) and dried in air. It can be shown that the obtained precipitate is starting material 7. The filtrate is cooled in an ice bath and acidified with con. hydrochloric acid to form a white precipitate that is filtered off, washed with water (2 x 25 mL) and dried in air. This product agrees in its spectral and analytical data with the structural assignment for 8, hence it is taken in the next step without further purification. Yield: 40.0 g (62%);m.p. 200-201°, lit. 198-201°. Recovered starting material: 20.5 g (37.0%).

4aH-r,1H-trans,10bH-cis,4H-trans,1-Acetylamino-4-bromo-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydrobenzo[b,d]pyrone-6 (9): To a well-stirred suspension of acetylamino carboxylic acid 8 (145.3 g, 0.48 mol) in tetrahydrofuran (2 L) powdered N-bromosuccinimide (89.0 g, 0.497 mol) is added in a single portion. Most of the solid dissolves, and a crystalline material begins to separate. After stirring for 1 hour the reaction mixture is cooled in an ice bath, then the precipitate is collected by filtration, washed with cold tetrahydrofurane, and dried. Yield: 175.5 g (93.3%); m.p. 265-267°, lit. 270°.

4aH-r,1H-trans,10bH-cis,1-Acetylamino-8,9-methylenedioxy-1,2,4a,10b-tetrahydrodibenzo[b,d]pyrone-6 (10): A mixture of acetylaminobromolactone 9 (456.1 g, 1.163 mol) and DBU (182 g, 1.2 mol) in pyridine (5.0 L) is kept at reflux for 8½ hours under anhydrous conditions. After cooling overnight a crystalline material precipitates. The solid is filtered off and slurred with water for 10 minutes, filtered, and air-dried. The filtrate is evaporated to dryness at oil vacuum pressure, and the solid residue is slurred in water for 10 minutes, filtered, air-dried, and combined with the previous precipitate. Yield: 339.0 g (93.7%); m.p. 270-272°, lit. 263-267°.

4aH-r,1H-trans,1-Hydroxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)-phenanthridone (11): A mixture of acetylaminolactone 10 (60.0 g, 0.193 mol) and ethanol (150 mL) is treated with a solution of NaOH (30 g) in water (150 mL), then the reaction is heated to 90-95°C, and kept at that temperature for 8 hours. A small amount of water is added periodically to dissolve a solid that separates. The reaction mixture is allowed to cool to room temperature overnight, the precipitated solid is collected by filtration, washed with water and dried in air. The filtrate is acidified with concentrated hydrochloric acid, the resulting precipitate is filtered, washed with water (3 x 100 mL) and dried in air. Both precipitated solids are found to be identical therefore they are combined, however, NMR-analysis indicates the presence of substantial amounts of starting material. The product is redissolved in 20% sodium hydroxide (300 mL), kept at 120° for five hours, cooled, and reprecipitated. Repeating the procedure five times the obtained product does not show any more contamination. Yield: 94.0 g; m.p. 260-280° (dec.), lit. 265-280° (dec.).

4aH-r,1H-trans,1-(2'-Tetrahydropyranyloxy)-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (12): To a suspension of hydroxyphenanthridone 11 (50 g, 0.193 mol) in dichloromethane (1.5 L) is added dihydropyran (70 mL, 0.857 mol) and p-toluene sulfonic acid (8.0 g). The reaction mixture is stirred for 72 hours with intermittent warming to 35°. The undissolved starting material is removed by filtration and the filtrate is washed with saturated sodium bicarbonate solution (2 x 500 mL) and water (500 mL). The organic layer is dried with sodium sulfate and evaporated to form a solid. Ethanol (25 mL) and ether (500 mL) are added to the solid and is kept overnight at room temperature. The solid is filtered, washed with ether (2 x 200 mL) and air-dried to yield a white crystalline material. Yield: 54.0 g; m.p. 218°.

4aH-r,1H-trans,1-(2'-Tetrahydropyranyloxy)-2,3-epoxy-8,9-methylenedioxy-1,4,4a,10b-tetrahydro-6(5H)phenanthridone (13): Phenanthridone derivative 12 (82.0 g, 0.239 mol) and 3-chloroperoxybenzoic acid (82.0 g, 0.478 mol) are dissolved in methylene chloride (1.4 L) and stirred for two days. The organic solution is washed with saturated sodium bicarbonate solution (2 x 600 mL) and water (2 x 500 mL), dried over sodium sulfate and concentrated under reduced pressure to a volume of approx. 75 mL. Ether (200 mL) is added and the resulting precipitate is collected by filtration and washed with ether (100 mL). Yield: 71.1 g; m.p. 243°, lit. 250°.

4H-r,1H-trans,2H-cis,10bH-trans,1-(2'-Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)-phenanthridone (14): To a suspension of diphenyldiselenide (25.3 g, 0.081 mol) in anhydrous ethanol (500 mL) is added sodium borohydride (6.5 g, 0.17 mol) in small portions, while controlling the temperature with an ice bath. To the clear solution epoxide 13 (54.0 g, 0.15 mol) is added in one portion, then the reaction mixture is maintained at reflux temperature for two hours. Subsequently, the volume of the reaction solution is reduced to 250 mL by evaporation under reduced pressure, tetrahydrofurane (750 mL) is added, and the temperature is adjusted to about 3° in an ice bath. Hydrogen peroxide (30 wt. %, 250 mL, 2.2 mol equiv.) is slowly added while stirring, during which time a white solid precipitates. The reaction mixture is heated to reflux temperature, and reflux is maintained for 7 hours during which time all the solid dissolves and the reaction mixture becomes dark in color. Upon cooling, water (2 L) is added and the mixture is extracted with ethyl acetate (3 x 700 mL). The organic phase is washed with water (2 x 500 mL),dried over sodium sulfate, and upon evaporation of the solvent a white solid is obtained, which is washed with ether (2 x 200 mL). Yield: 38.5 g; m.p. 230-235°; lit. 232°.

4aH-r, 1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1-(2'-Tetrahydropyranyloxy)-2,3,4-trihydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (15): To a solution of N-methylmorpholine-N-oxide (16.0 g, 0.137 mol) in t-butanol (50 mL), acetone (50 mL), and water (20 mL) osmium tetroxide (260 mg, 1 mmol) is added. To this reaction medium is added a solution of intermediate 14 (28.8 g, 80 mmol) in t-butanol (700 mL) over a 10 minute period, followed by continued stirring for 48 hours. Decolorizing carbon is added to the dark solution, and after stirring for 3 hours at room temperature the reaction mixture is filtered through a Celite bed. The obtained pale-yellow solution is evaporated to an oil under reduced pressure. The residue is triturated with ethanol (25 mL), and the resulting crystalline material is collected by filtration. The solid is suspended in water (100 mL), stirred for one hour and filtered. The off-white solid is washed with water, and dried in air to give trihydroxy compound 15. Yield: 20.0 g; m.p. 222-223°.

4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1-Hydroxy-2,3,4-triacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone(16): A mixture of trihydroxy compound 15 (18.6 g, 0.047 mol), pyridine (200 mL) and acetic anhydride (200 mL) is stirred at room temperature overnight. Acetic anhydride and pyridine are evaporated under reduced pressure, followed by a distillation with ethanol to remove all pyridine. The residual material is soaked in ethanol (50 mL) and chilled. The crystalline material is collected by filtration, washed with ethanol (50 mL) and air-dried. Yield: 22.0 g; m.p. 275°C.

This material is suspended in ethanol (500 mL), p-toluene sulfonic acid (500 mg) is added, and the mixture is kept at reflux for 2 hours. The crystalline precipitate that forms is filtered off, washed with cold ethanol (2 x 25 mL) and dried in air. Yield: 15.0 g; m.p. 303-304°.

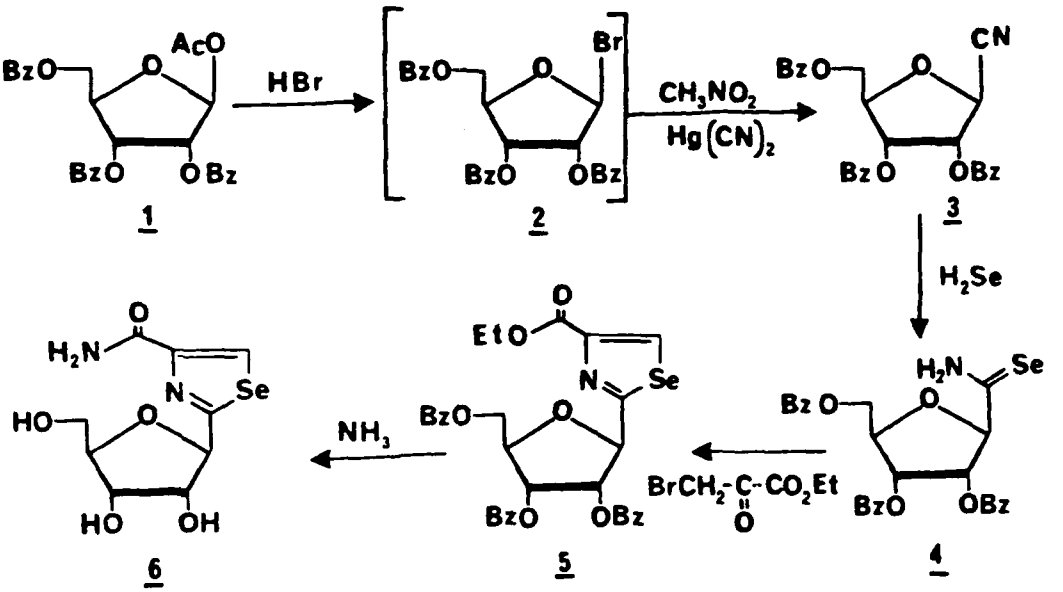
Lycoricidine Triacetate (4aH-r,1H-trans,2H-cis,3H-trans,4H-trans,-10bH-trans,2,3,4-Triacetoxy-8,9-methylenedioxy-2,3,4,4a-tetrahydro-6(5H)-phenanthridone) (17): Intermediate 16 (7.0 g, 18 mmol) is dissolved in pyridine (70 mL) with stirring, and upon cooling thionyl chloride (10 mL) is added over a 20 minute period. Stirring is continued overnight while the reaction mixture is allowed to warm up to room temperature. Dichloromethane (500 mL) was added to the reaction, and the mixture is washed with water twice, with 10% hydrochloric acid (2 x 250 mL) and water (2 x 500 mL). The organic phase is dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield a white solid. Upon trituration with methanol (40 mL) the crystalline material is filtered, washed with methanol (2 x 20 mL) and dried in air. This product is recrystallized from dichloromethane-methanol 1:1 (50 mL) to give pure lycoricidine triacetate. Yield: 4.0 g; m.p. 266-268° (decomp.).

From the mother liquor a second crop can be obtained to yield 2.0 g of final product.

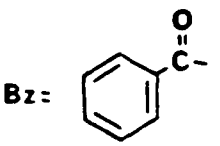
Remark: Compound 15 and 16 are unreported in the literature,[7] therefore they are fully characterized by analytical and spectral data, which are in agreement with structural assignments. The applied modifications produce better results than the reported procedure, therefore a communication has been submitted to *Synthesis* for publication (See addendum at the end of this report).

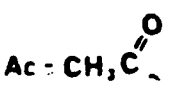
H. 2-β-D-Ribofuranosyl-selenazo-4-carboxamide, AVS 253

Synthetic Procedure:[8]



AVS 253





2,3,5-Tri-O-benzoyl-ß-D-ribofuranosyl-1-carbonitrile (3): Hydrogen bromide gas is injected into a solution of acetyl-tribenzoylribose 1 (252 g, 0.5 mol) in toluene (1000 mL) at 0-5°C until saturation. Upon reaching room temperature, the excess hydrobromide is removed by injecting a stream of nitrogen for one hour. The solvent is removed by distillation under reduced pressure, and the residue is dissolved in nitromethane (600 mL). Mercuric cyanide (240 g, 0.95 mol) is added, and after stirring for 20 hours the insoluble material is collected by filtration and washed with ethyl acetate. The combined organic layers are evaporated to dryness, dissolved in ethyl acetate (1000 mL) and washed with a sodium hydrosulfide solution (100 g in 1 L water). The aqueous phase is washed with ethyl acetate, the organic layer is combined with the initial ethyl acetate layer, and dried over anhydrous sodium sulfate. After treatment with charcoal (30 g) the mixture is filtered through a Celite bed, the solvent is evaporated under reduced pressure, and the resulting syrup is dissolved in ethanol (2 L). Upon stirring overnight, the formed crystalline material is collected by filtration. After recrystallization from ethanol, 151 g (64%) of the cyanosugar 3 is obtained. m.p. 77-79° (lit. 78-80) TLC: Silica gel - benzene/ethylacetate 4:1, one spot at Rf 0.85.

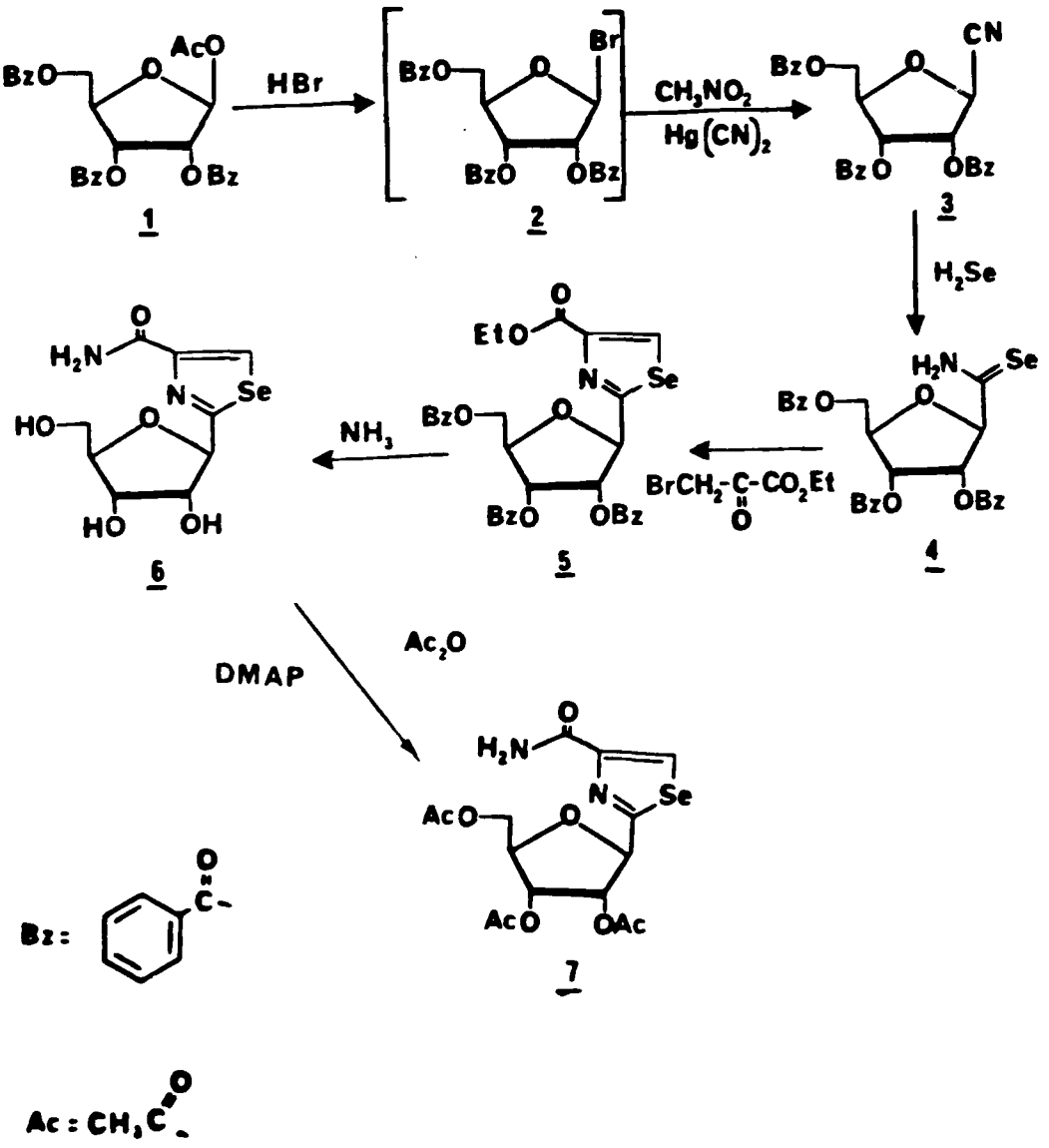
2-β-D-Ribofuranosyl-4-selenazole carboxamide (6): Cyanosugar 3 (315 g, 0.668 mol) and dimethylamino pyridine (6.3 g) are dissolved in absolute ethanol with gentle warming to 45°. The solution is cooled to room temperature, and after purging with argon gaseous hydrogen selenide (56 g, 0.7 mol) is injected over a 90 minute time span. After TLC indicates the completion the solution is again purged with argon, followed by addition of ethyl bromopyruvate 90% (151.0 g, 0.7 mol). Upon stirring for 45 minutes the solution is neutralized with 5% sodium bicarbonate solution (2 L) and filtered through a celite bed which is washed with ethanol (1 L) and dichloromethane (1 L). After evaporation of the filtrate under reduced pressure the residue is dissolved in dichloromethane (2 L), then the solution is washed with sodium bicarbonate solution (25 g in 750 mL water), and with water.

Drying over magnesium sulfate and evaporating the solvent leaves crude carboxylate 5 (440 g), which is dissolved in methanol (1400 mL) and transferred to a steel bomb. After cooling the contents to 0° ammonia gas is injected until saturation is reached. The bomb is sealed, heated to 115° for 6 hours, cooled, and vented. The reaction mixture is concentrated under reduced pressure, distilled twice with additional methanol (2 x 300 mL), and the obtained syrup is dissolved in water at 50°. The aqueous phase is extracted with ethyl acetate, treated with charcoal (30 g) and filtered through a Celite pad. Upon evaporation of the water a gummy residue is obtained, which is crude nucleoside 6 of approximately 90% purity, as indicated by TLC. The gum is purified by repeated separations on a silica gel column (6 x 50 cm, silica gel 200-425 mesh), with dichloro methane/methanol 4:1 (6 L) as eluant.

After combining the fractions containing 6, concentrating the volume, and placing the solution in the freezer overnight, only a small amount of crystals forms. The mother liquor again has to be submitted to column chromatographic purification to obtain another crop. A total of four crystallizations produces 37.6 g of pure crystalline material, and the mother liquor is left to allow for additional crystal formations. m.p. 130-132°; lit. 129-131.

I. 2',3',5'-Tri-O-acetyl-2-β-D-ribofuranosyl-selenazo-4-carboxamide, AVS 253 TA

Synthetic Procedure:

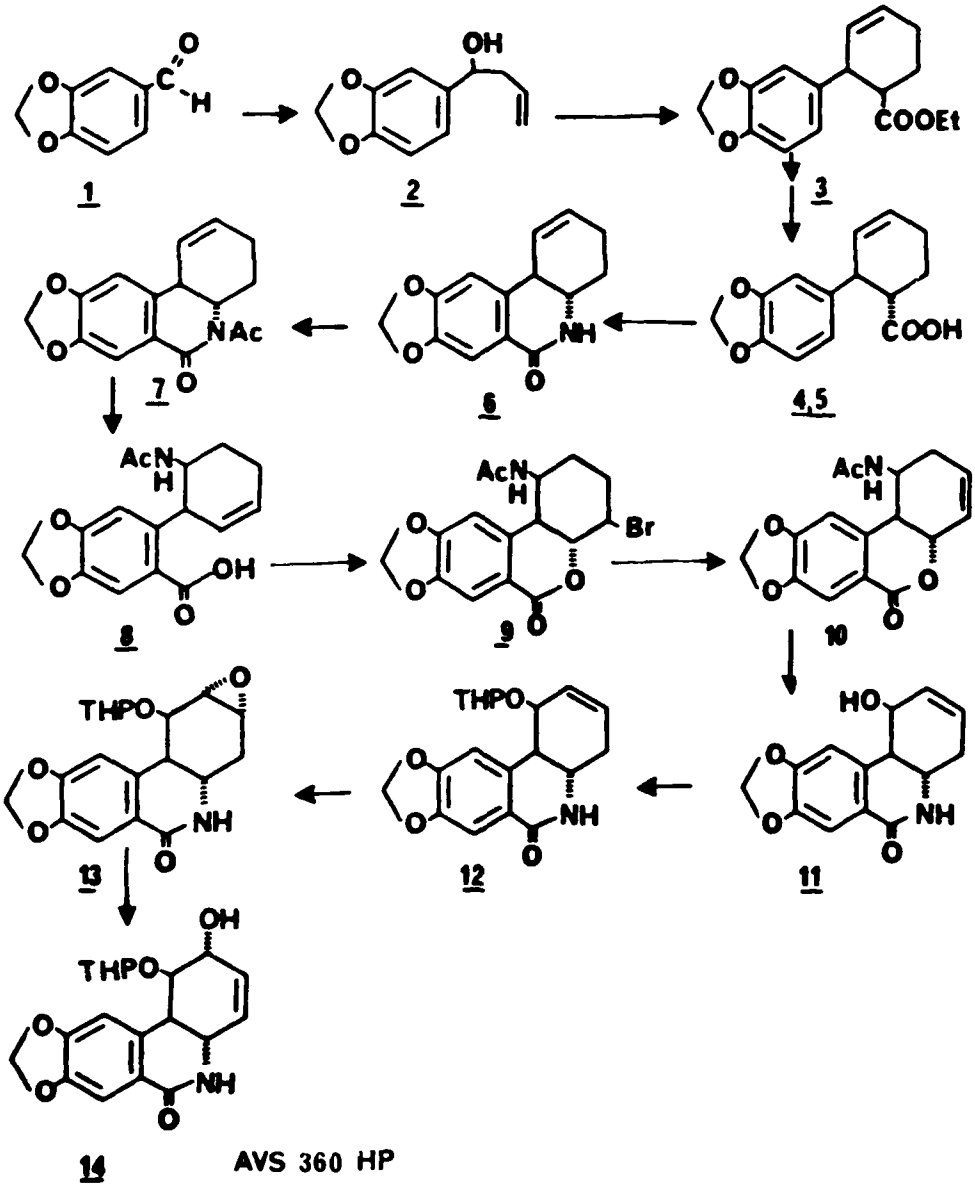


AVS 253 TA

The residue recovered from the mother liquor of preparation AVS 253, which contains about 80% selenazole carboxamide 6 (33 g) is combined with acetic anhydride (350 mL). Dimethylamino pyridine (1.0 g) is added and the mixture is stirred at room temperature for 60 hours. The unreacted material is filtered off, the solution is concentrated to an oil, dissolved in dichloromethane, and washed with water (500 mL), sodium bicarbonate solution (2 x 500 mL) and water (500 mL). The organic phase is dried over magnesium sulfate, evaporated, and the semi-solid residue is recrystallized from water/ethanol 10:1 (400 mL) to give crystalline acetyl derivative 7. Yield 13.5 g (36%), m.p. 119-120°, lit. 117-119°.

K. 4H-r,1H-trans,2H-cis,10bH-trans,1-(2'-Tetrahydropyranyloxy)-2-hydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)phenanthridone (AVS 360 HP)

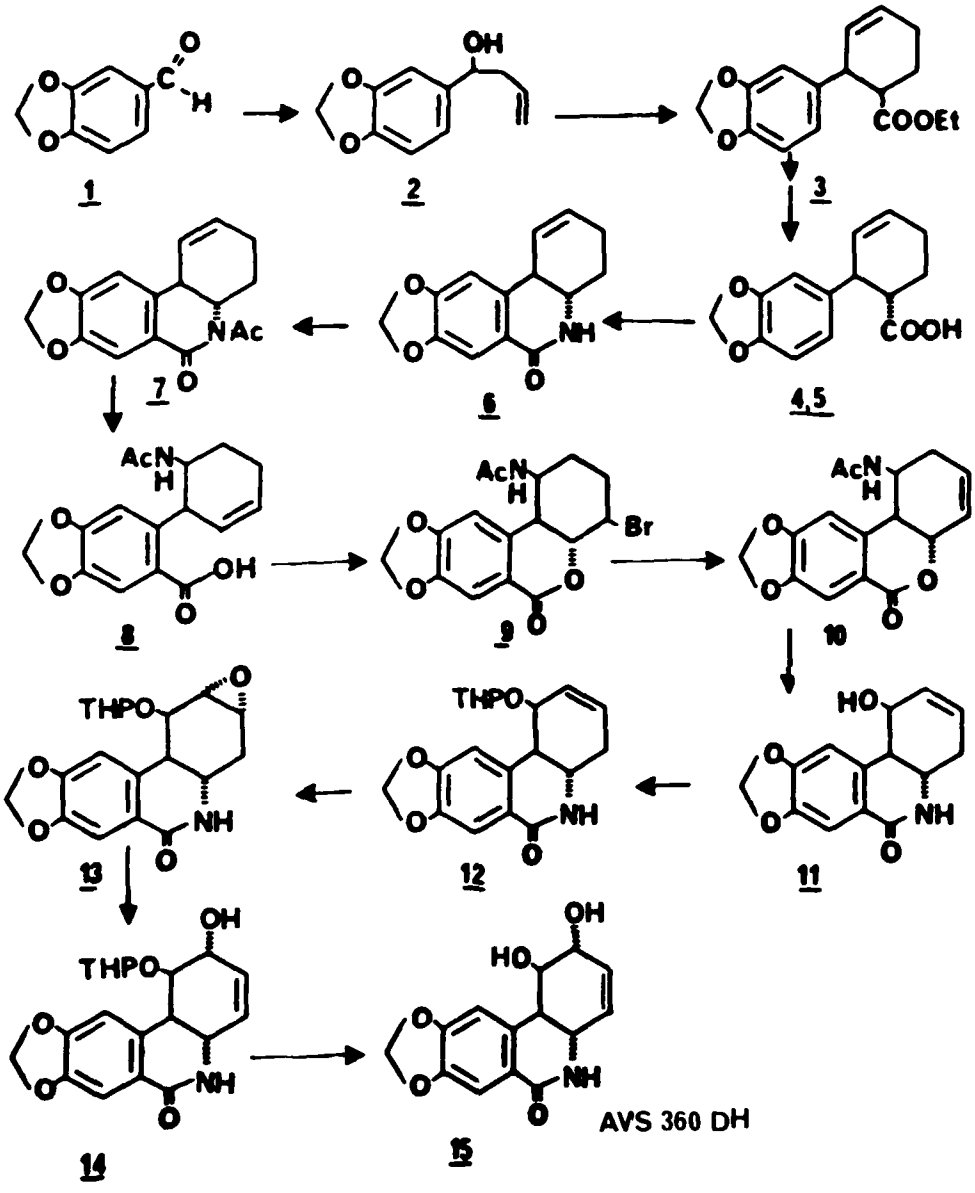
Synthetic Procedure:[6]



Structure AVS 360 HP represents an intermediate, obtained during the synthesis of Lycoricidine triacetate (AVS 360), and after purification several grams were submitted to USAMRIID.

L. 4a-r,1H-trans,2H-cis,10bH-trans,1,2,-Dihydroxy-8,9-methylenedioxy-1,2,4a,10b-tetrahydro-6(5H)phenanthridone (AVS 360 DH)

Synthetic Procedure:[7]

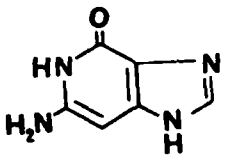


Compound AVS 360 DH is obtained by deblocking the tetrahydropyranyl protecting group of AVS 360 HP, and the product was submitted to USAMRIID.

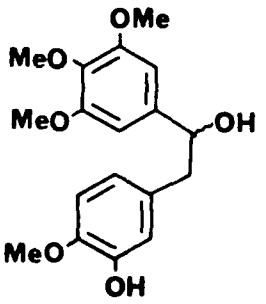
V. DISCUSSION OF UNCOMPLETED TARGET COMPOUNDS

List and Structures of Compounds in Progress:

AVS 272	3-Deazaguanine
AVS 353	Combretastatin



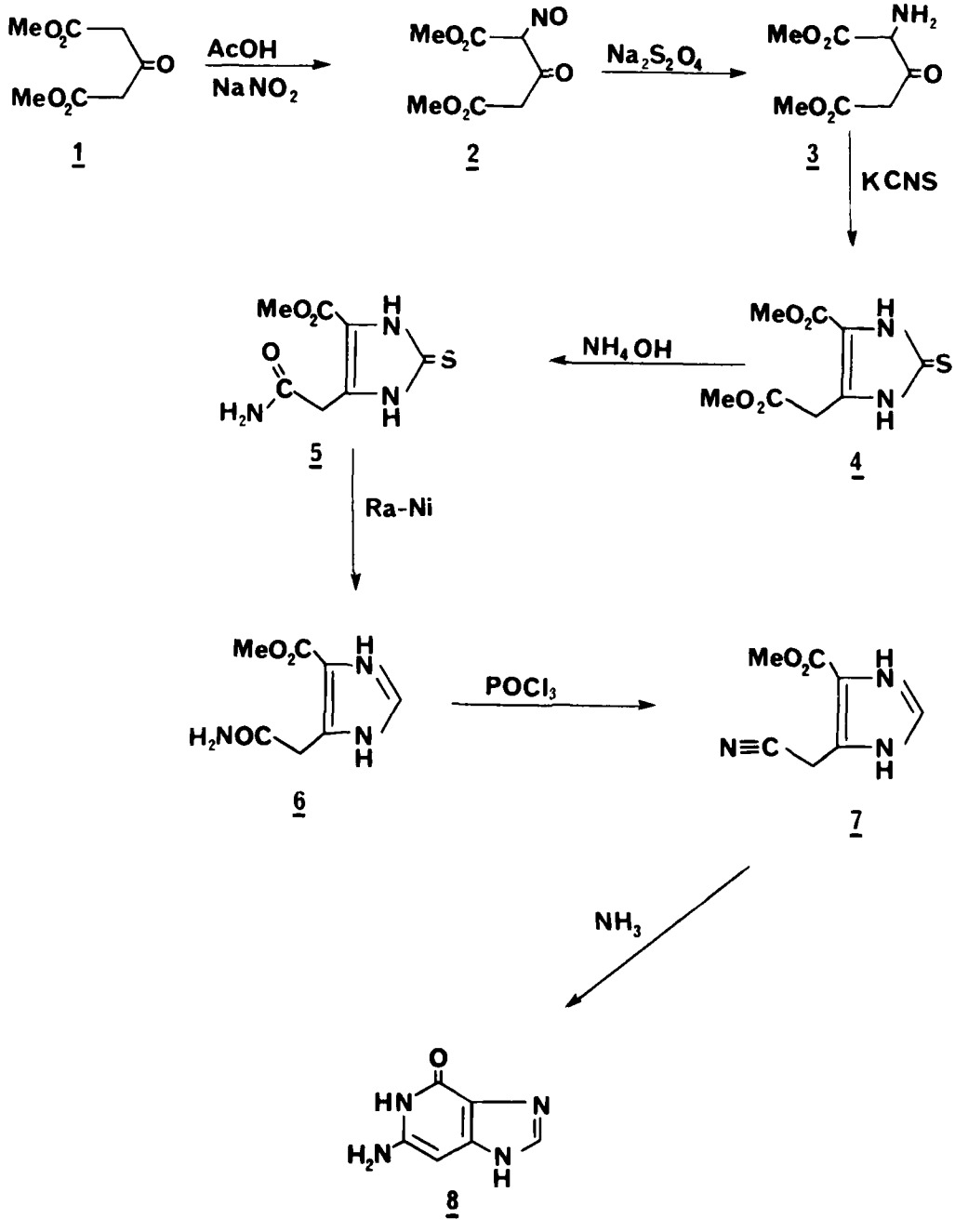
AVS 272



AVS 353

M. 3-Deazaguanine (AVS 272)[9,10]

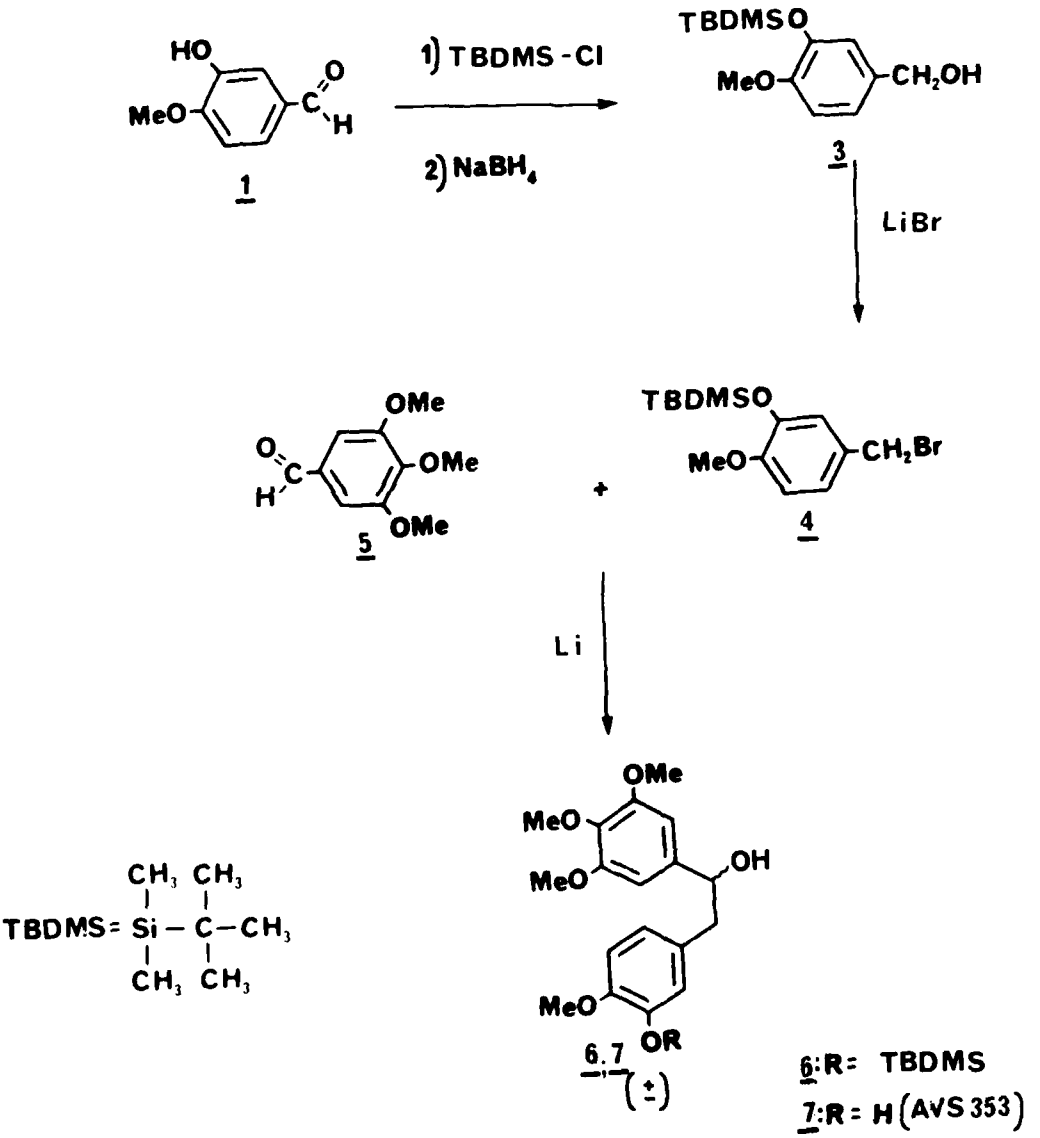
Presently, a sufficient amount of nitrile 7 has been prepared to allow for the cyclization reaction as the final step. A smaller quantity of the final product was prepared, and presently attempts are made to scale up this final reaction step.



AVS 272

N. Combretastatin (AVS 353)[11]

The synthesis of Combretastatin, intended to be accomplished by the shown pathway, was initiated by preparing a sufficient amount of intermediate 4. Presently, attempts are being made to couple intermediate 4 with aldehyde 5. This coupling reaction does not produce favorable yields upon scale-up, therefore several modifications are being investigated.



VI. **REFERENCES**

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VII. ACKNOWLEDGMENTS

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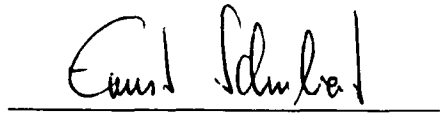
Report Submitted By:
Pharm-Eco Laboratories, Inc.



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VIII. APPENDIX

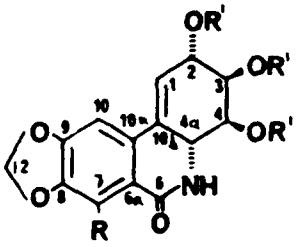
Research Communication Submitted for Publication

Improved Synthesis of Lycoricidine Triacetate

Bheemarao G. Ugarkar, Jay DaRe, and Ernst M. Schubert*;
Pharm-Eco Laboratories, Inc., 2355 Chain Drive, Simi Valley, California 93065.

The synthesis of lycoricidine triacetate by a modified pathway is described. In this preparation catalytic amounts of osmium tetroxide are used to stereospecifically introduce two hydroxyl groups, rendering the title compound via two novel intermediates.

Lycoricidine (1) and lycoricidinol (2), two constituents found in *Amaryllidaceae* plants, show strong growth-inhibiting action in the rice seedling test, and they exhibit anti-tumor activity against Ehrlich carcinoma[1]. Such antimitotic behavior prompted further investigations to establish their configurations and conformations[1], which were subsequently confirmed by total synthesis[2,3].

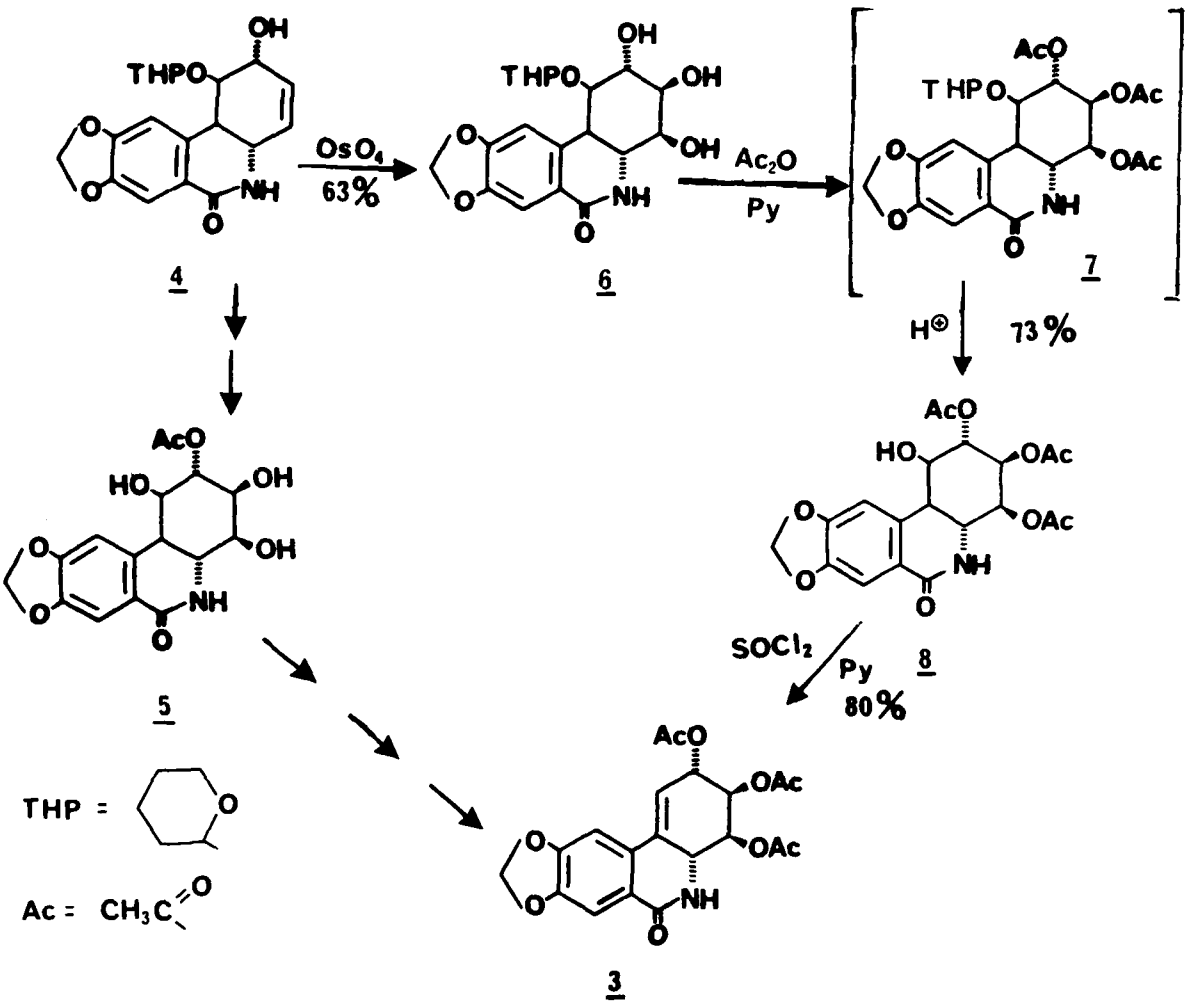


1: R=H; R'=H

2: R=OH; R'=H

3: R=H; R'=CH_3CO

During recent *in vitro* testing of lycoricidine triacetate (3) it displayed antiviral activity[4], and a proposed *in vivo* study necessitated the preparation of a larger quantity of 3. The isolation of lycoricidine from plant material is impractical since its abundance in fresh bulbs of *Lycoris* is about 3.2×10^{-4}%[1], while a reported synthesis produced lycoricidine in a 21-step procedure with 1.5% overall yield, starting with piperonal[3]. After establishing the desired phenanthridone intermediate 4 following a twelve step procedure, acetylation and removal of the tetrahydropyranyl ether is followed by stereospecific *cis*-hydroxylation with equimolar amounts of osmium tetroxide to yield intermediate 5.



Synthetic Scheme

The two *cis*-hydroxyl groups in 5 are protected as the isopropylidene ketal to allow for the introduction of a double bond between C-1 and C-10b by dehydration. Acetylation, following removal of the isopropylidene group, renders lycoricidine triacetate in about 26% yield, based on intermediate 4.

The improved preparation of 3, as shown in the synthetic scheme, starts with the direct *cis*-hydroxylation of 4 by utilizing only catalytic amounts of osmium tetroxide, according to a method described by VanRheenen *et al.*[5] to yield trihydroxy compound 6. The absence of isomers in the oxidation mixture prior to work-up, as indicated by thin-layer chromatography, attests to the applicability of osmium tetroxide as a highly stereoselective reagent.

After acetylation of the three hydroxyl groups in 6, the resulting tetrahydropyranyl ether 7 is hydrolyzed without prior purification to render intermediate 8, which upon dehydration yields lycoricidine triacetate in 37% overall yield, starting with 4.

Spectral and analytical data confirm the structural assignments of 3 and, based on 400 MHz ^{1}H NMR, ^{13}C-NMR, COSY and HOMCOR spectra, the spatial arrangement at the four chiral carbons is in agreement with the absolute structure assigned to lycoricidine. Two different melting points have been reported for lycoricidine triacetate, depending on its origin[3], but such a difference could be a result of the mode of recrystallization to obtain a product of differing crystal structure. Hydrolysis of the prepared lycoricidine triacetate, as described by Ohta and Kimoto[3], produces lycoricidine (1), whose spectral and analytical data agree with the reported values for lycoricidine of natural origin.

Presently, lycoricidine triacetate (3) is being screened for its *in vivo* antiviral activity, and if proven useful as an antiviral agent, it can readily be prepared according to the reported modifications. They make the procedure economically more attractive for scale-up work because they reduce the number of required steps thus improving the overall yield, while significantly reducing the hazards of handling and disposing of large amounts of highly toxic osmium oxides[6].

Experimental

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. In addition to the standard nuclear magnetic resonance spectra, taken on a Varian EM 390 spectrometer, 400 MHz ^{1}H NMR, ^{13}C-NMR, attached proton test (ATP), homonuclear correlation (COSY), and heteronuclear correlation (HETCOR) spectra were recorded by SRI International, Menlo Park, California. Infrared spectra were obtained on a Beckman AccuLab 2, and ultraviolet spectra were recorded on a Beckman Model 25 spectrophotometer. Mass spectral analyses were taken on a Varian Model 311 A spectrometer by Dr. K. H. Schram, College of Pharmacy, University of Arizona, Tucson, AZ. Elemental analyses were performed by MHW Laboratories, Phoenix, Arizona. Thin-layer chromatography was run on Silica Gel GF plates (Analtech, Newark, Del.) where the products were visualized by UV-absorbance, or by iodine stains. All solvents used were of reagent grade.

4aH- ,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1-(2-Tetrahydropyranyloxy)-2,3,4-trihydroxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (6): To a solution of N-methylmorpholine-N-oxide[7] (16.0 g, 0.137 mol) in t-butanol (50 mL), acetone (50 mL), and water (20 mL) osmium tetroxide (260 mg, 1 mmol) is added. To this reaction medium is added a solution of phenanthridone derivative 4[2] (28.8 g, 80 mmol) in t-butanol (700 mL) over a 10 minute period, followed by continued stirring for 48 hours. Decolorizing carbon is added to the dark solution, and after stirring for 3 hours at room temperature the reaction mixture is filtered through a Celite bed. The resulting pale-yellow solution is evaporated under reduced pressure to leave an oil. This oil is triturated with ethanol (25 mL), and the resulting crystalline material is collected

by filtration. The solid is suspended in water (100 mL), stirred for one hour and filtered. The off-white solid is washed with water, and air-dried to give trihydroxy compound 6. Yield: 20.0 g (63%); m.p. 222-223°.

$C_{19}H_{23}NO_8$	Calc.	C	58.01	H	5.89	N 3.56
(393.4)	Found		58.42		5.96	3.62

^{1}H NMR (DMSO-d_6/TMS int.): δ=1.4 (s, br 9H, tetrahydropyrane); 3.1 - 4.5 (m, 8H, 5 aliph. H + 3OH); 4.9 (s, 1H, H-4a); 6.15 (d, 2H, J=2.5 Hz, -CH_2-); 6.8 (s, 1H, NH); 7.05 (s, 1H, H-10) 7.4 (s, 1H, H-7).

IR (KBr): 3600-3200 (br), 2970, 1665, 1610, 1495, 1460, 1390, 1355, 1260, 1075, 1035 cm^{-1}.

TLC: Chloroform/methanol 6:1; Rf, 0.60.

4aH-,1H-trans,2H-cis,3H-trans,4H-trans,10bH-trans-1-Hydroxy-2,3,4-triacetoxy-8,9-methylenedioxy-1,2,3,4,4a,10b-hexahydro-6(5H)phenanthridone (8): A mixture of trihydroxy compound 6 (18.6 g, 0.047 mol), pyridine (100 mL) and acetic anhydride (200 mL) is stirred at room temperature overnight. Acetic anhydride and pyridine are evaporated under reduced pressure, followed by evaporation with ethanol to remove traces of pyridine. The residual material is soaked in ethanol (50 mL) and chilled. The resulting crystalline material is collected by filtration, washed with ethanol (50 mL) and air-dried. Yield: 22.0 g; m.p. 275°.

Without further characterization intermediate 7 is suspended in ethanol (500 mL), p-toluenesulfonic acid (500 mg) is added, and the mixture is kept at reflux for 2 hours. Upon cooling the crystalline material is filtered, washed with cold ethanol (2 x 25 mL) and dried in air. Yield: 15.0 g (73%); m.p. 303-304°.

$C_{20}H_{21}NO_{16}$	Calc.	C	55.17	H	4.86	N 3.21
435.4	Found		54.95		4.90	3.13

^{1}H NMR (DMSO-d_6/TMS int.): δ=2.05 (s, 6H, 2 acetyl); 2.15 (s, 3H, 1 acetyl); 3.1 - 5.5 (m, 7H, aliph.); 6.15 (s, 2H, -CH_2-); 6.9 (s, 1H, NH); 7.35 (s, 1H, H-10) 7.9 (s, 1H, H-7).

IR (KBr): 3460, 3180, 3070, 2965, 1740, 1670, 1480, 1450, 1360, 1250, 1215, 1040, 920 cm^{-1}.

TLC: Chloroform/methanol 6:1; Rf, 0.75.

Lycoricidine Triacetate (4aH- ,1H-trans,2H-cis,3H-trans,4H-trans,-10bH-trans,2,3,4-Triacetoxy-8,9-methylenedioxy-2,3,4,4a-tetrahydro-6(5H)-phenanthridone) (3): Intermediate 8 (7.0 g, 18 mmol) is dissolved in pyridine (70 mL) while stirring, and upon cooling thionyl chloride (10 mL) is added over a 20 minute period. Stirring is continued overnight while the reaction mixture is allowed to warm up to room temperature. Dichloromethane (500 mL) is added to the reaction mixture, and the solution is washed with water (2 x 500 mL), with 10% hydrochloric acid (2 x 250 mL) and water (2 x 500 mL). The organic phase is dried over anhydrous sodium sulfate and evaporated under reduced pressure to yield a white solid. Upon trituration with methanol (40 mL) the crystalline material is filtered, washed with methanol (2 x 20 mL) and dried in air. This product is recrystallized from dichloromethane-methanol 1:1 (50 mL) to give pure lycoricidine triacetate. From the mother liquor a second crop is obtained to give a total yield of 6.0 g (80%); m.p. 266-268° (decomp.).

$C_{20}H_{19}NO_9$	Calc.	C 57.55	H 4.59	N 3.35
417.3	Found	57.46	4.50	3.36

^{1}H NMR ($CDCl_3$/TMS int.): δ=2.09 (s, 3H, acetyl), 2.11 (s, 3H, acetyl), 2.15 (s, 3H, acetyl), 4.65 (d, J=10 Hz, 1H, H-4a), 5.2 - 5.4 (m, 3H, H-2, H-3, H-4), 6.10 (s, 3H, H-1, H-12), 7.00 (s, 1H, H-10), 7.35 (s, 1H, NH), 7.50 (s, 1H, H-7).

^{13}C NMR ($CDCl_3$/TMS int.) δ=21.0 (3C, acetyl), 50.1 (C-4a), 68.2 (C-4), 68.6 (C-3), 71.2 (C-2), 102.0 (C-12), 103.4 (C-10), 107.5 (C-7), 117.0 (C-1), 122.5 (C-10b), 130.3 (C-10a), 134.1 (C-6a), 149.2 (C-9), 151.7 (C-8), 164.3 (C-6), 169.5 (C=O), 169.7 (C=O), 170.4 (C=O).

The ultraviolet, infrared, and mass spectral data all agree with the reported values[3].

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4) Initial tests were performed under the direction of Dr. Peter Canonico, U.S. Army Medical Research Institute for Infectious Diseases, Fort Detrick, Frederick, MD.

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